
PHASE II STUDY OF NONMYELOABLATIVE PERIPHERAL BLOOD STEM CELL TRANSPLANT WITH HIGH-DOSE POSTTRANSPLANTATION CYCLOPHOSPHAMIDE IN HEMATOPOIETIC MALIGNANCIES INCLUDING THOSE THAT ARE CHALLENGING TO ENGRAFT

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Study Product: Cyclophosphamide

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Mesna, commercial
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Mycophenolate, commercial
Tacrolimus, commercial
Sirolimus, commercial

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List of Abbreviations

CLL	chronic lymphocytic leukemia
CMMML	chronic myelomonocytic leukemia
CNI	calcineurin inhibitor
CrCl	creatinine clearance
Cy	cyclophosphamide
GVHD	Graft-versus-host-disease
GVT	grat-versus-tumor
HSCT	Hematopoietic Stem Cell Transplantation
IMWG	International Myeloma Working Group
IST	immunosuppressive therapy
MDS	myelodysplastic syndrome
MHC	major histocompatibility complex
MM	multiple myeloma
MMF	mycophenolate mofetil
MPD	myeloproliferative disease
NRM	nonrelapse mortality
OS	overall survival
PBSC	peripheral blood stem cell
PFS	Progressive free survival
PRES	Posterior Reversible Encephalopathy Syndrome
TBI	Total Body Irradiation

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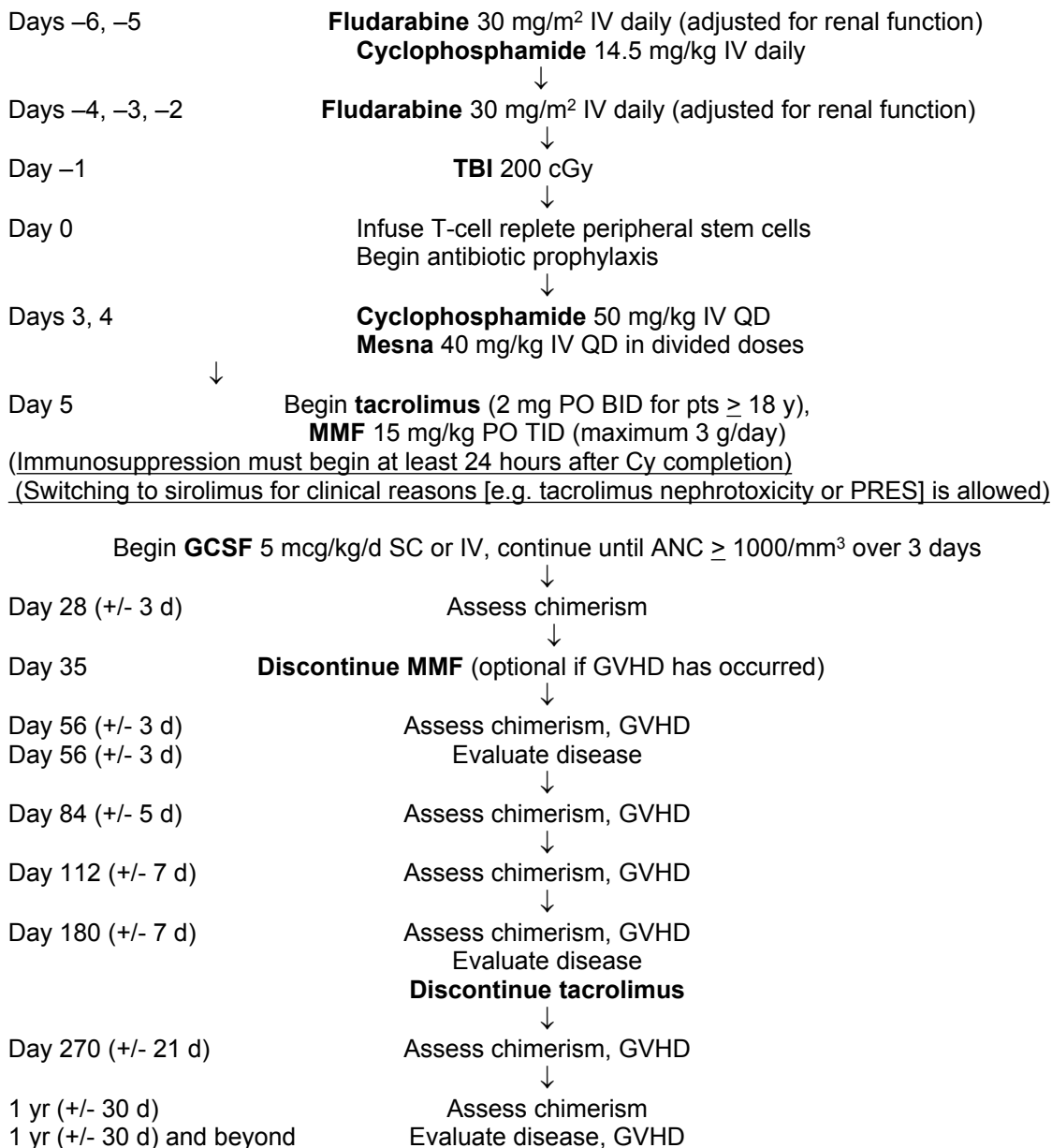
Title	Phase II study of nonmyeloablative peripheral blood stem cell transplant with high-dose posttransplantation cyclophosphamide in hematopoietic malignancies including those that are challenging to engraft
Protocol Number	s17-00042
Phase	II
Methodology	Single arm open label Phase II clinical trial of peripheral blood stem cell transplantation and posttransplantation cyclophosphamide therapy from full and haploidentical related donors in hematological malignancies, including those difficult to engraft
Study Duration	2.5 years (1 year after enrollment of last patient)
Study Center(s)	Laura & Isaac Perlmutter Cancer Center at NYU Langone
Objectives	<p>Overall Objective: In nonmyeloablative, related or unrelated donor, partially HLA-mismatched or HLA-mismatched transplant with post-grafting immunosuppression that includes high-dose cyclophosphamide and MMF, and standard tacrolimus (Day 5 through Day 180) evaluate the safety and feasibility of peripheral stem cells as a source of stem cells in hematopoietic malignancies, including those challenging to engraft.</p> <p>Primary Objective:</p> <p>Estimate the one year event (relapse, progression, or death) free survival (EFS) rate.</p> <p>Secondary Objectives:</p> <ol style="list-style-type: none"> 1. Estimate the cumulative incidences of severe acute grade III or higher GVHD, chronic GVHD (overall and by extent) 2. Estimate the cumulative incidence of systemic steroid initiation 3. Summarize the graft failure frequency 4. Summarize the kinetics of neutrophil and platelet recovery, and kinetics of donor chimerism in unsorted and CD3+ sorted peripheral blood 5. Summarize major toxicities and complications associated with the transplantation procedure selected toxicities <p>Exploratory Objectives:</p> <p>Explore the association between the amount of donor T cell chimerism at ~ Day 28 and patient/graft characteristics (e.g., prior therapies, graft cell dose) and transplantation outcomes (sustained engraftment, relapse or progression, GVHD)</p>
Number of Subjects	15 subjects

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<p>Dia⁹nosis and Main Inclusion Criteria</p>	<p>Eligibility for transplantation</p> <ol style="list-style-type: none"> 1. Presence of a suitable related, HLA-haploidentical or HLA-matched stem cell donor <ol style="list-style-type: none"> a. The donor and recipient must be identical at least one <u>allele</u> of each of the following genetic loci: HLA-A, HLA-B, HLA-Cw, HLA-DRB1, and HLA-DQB1. A minimum match of 5/10 is therefore required for related donors, and will be considered sufficient evidence that the donor and recipient share one HLA haplotype. Unrelated donors will be 10/10. 2. Eligible diagnoses: <ol style="list-style-type: none"> a. Myelodysplastic syndrome (MDS) including chronic myelomonocytic leukemia [CMML] b. SLL or CLL with 17p deletion, or with progression < 6 months after second or greater treatment regimen. c. T-cell PLL in PR or better prior to transplantation d. Interferon- or tyrosine kinase-refractory CML in first chronic phase, TKI-intolerant CML in first chronic phase e. Philadelphia chromosome negative myeloproliferative disease (including myelofibrosis) f. Multiple myeloma or plasma cell leukemia with a PR g. Hematologic malignancy in complete or partial remission with minimal residual disease (MRD) detectable or non-detectable by conventional cytogenetics, FISH, flow cytometry, molecular testing or PET/CT imaging. 3. No active extramedullary leukemia <p>Donor eligibility</p> <ol style="list-style-type: none"> 1. Donors must be either: <ol style="list-style-type: none"> a. HLA-haploidentical or HLA-identical relatives of the patient based on allele or allele group level typing as defined in Section 3.1. 2. Medically fit to and willing to donate 3. Lack of recipient anti-donor HLA antibody <p><u>Note:</u> In some instances, low level, non-cytotoxic HLA specific antibodies may be permissible if they are found to be at a level well below that detectable by flow cytometry. This will be decided on a case-by-case basis by the PI and one of the immunogenetics directors. Pheresis to reduce anti-HLA antibodies is permissible; however eligibility to proceed with the transplant regimen would be contingent upon the success of the desensitization.</p> 4. Has not donated blood products to patient
<p>Study Product, Dose, Route, Regimen</p>	<p>cyclophosphamide</p>

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Schema^a

^a See Section 5.3 for complete dosing instructions, and Section 7.0 for required evaluations.

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1 Introduction

This document is a protocol for a human research study. This study is to be conducted in accordance with US government research regulations, and applicable international standards of Good Clinical Practice, and institutional research policies and procedures.

This protocol has been modified from a Johns Hopkins Protocol “*PHASE II STUDY OF NONMYELOABLATIVE PERIPHERAL BLOOD STEM CELL TRANSPLANT WITH HIGH-DOSE POSTTRANSPLANTATION CYCLOPHOSPHAMIDE IN HEMATOPOIETIC MALIGNANCIES INCLUDING THOSE THAT ARE CHALLENGING TO ENGRAFT*” approved by the Johns Hopkins IRB 10/20/15. The Johns Hopkins Protocol has an arm in which they discontinue immunosuppression at Day 90 in some patients; this arm is not included in the current study.

Recent advances in allogeneic blood or marrow transplant (HSCT) platforms for hematologic malignancies have substantially lowered transplant-related morbidity both in the HLA-matched and partially HLA-mismatched settings. One of these major advances is the incorporation of high-dose posttransplantation cyclophosphamide (Cy) for prophylaxis of graft-versus-host-disease (GVHD) and graft rejection, as developed at Johns Hopkins.¹ This approach has made the use of mismatched donors, including haploidentical donors, possible. Historically, this approach has been used for HSCTs which have used marrow as the graft source. However, the HSCT field has been moving towards using peripheral blood stem cells (PBSC) as a graft source in nonmyeloablative allogeneic transplants based on data showing lower graft failure, progression free survival (PFS) and overall survival (OS) benefit.² Although transplants using PBSCs have higher rates of GVHD than those from marrow sources, the GVHD may translate into better outcomes in terms of OS and PFS.^{3,4} This might be particularly important for those diseases that are more challenging to engraft, including myelodysplastic syndrome (MDS), chronic lymphocytic leukemia (CLL), myeloproliferative diseases (MPD) and multiple myeloma (MM).

This study will therefore assess engraftment, GVHD, and overall survival in patients receiving PBSC from HLA haploidentical or matched donors using a nonmyeloablative conditioning regimen with GVH prophylaxis using post-transplant high-dose cyclophosphamide, tacrolimus, and mycophenolate mofetil. Our goal is to estimate one year EFS rate, and summarize the rates of GVHD in our population. We will compare our findings to published data of matched and haploidentical transplants, which have included bone marrow as a graft source and a range of post-transplant immunosuppression.

1.1 Background

Stem cell source

There has been a great deal of discussion on the importance of stem cell source on the risk of chronic graft-versus-host disease⁵⁻⁹. Several studies have addressed this issue in the related setting. Of the eight randomized trials published¹⁰⁻¹⁸ only one reported an statistically significant increase in grades II-IV acute graft-versus-host disease with the use of peripheral blood stem cells when compared to bone marrow (52 vs. 39%)¹⁶. Regarding chronic graft-versus-host disease, the results are as follows: 3 studies have shown an increase of chronic graft-versus-host disease with peripheral blood stem cells as opposed to bone marrow^{12,16,19}. One study showed a trend towards increase in chronic graft-versus-host disease with the use of peripheral blood stem cells¹⁹. A meta-analysis by Cutler et al. confirmed that both, acute and chronic graft-versus-host disease are more common after peripheral blood stem cells than bone marrow⁷. Registry data showed in pediatric patients that chronic graft-versus-host disease was more frequent (as well as higher mortality) after peripheral blood stem cells than after bone marrow⁸. In adults, chronic graft-versus-host disease is also more prevalent²⁰. Umbilical-cord stem cells also have been a source of grafts in children and young adults. As children tolerate mismatches better than adults, interpretation of risk in this group is difficult but it seems that the rate of chronic graft-versus-host disease is low for this stem cell sources, especially considering that almost all grafts are 1-3 antigen mismatches^{21,22}. In the unrelated setting, a clinical trial by the BMT CTN comparing bone marrow versus peripheral blood did not detect significant survival. Peripheral-blood stem cells may reduce the risk of graft failure (the overall incidence of graft failure in the peripheral-blood group was 3% [95% CI, 1 to 5], versus 9% [95% CI, 6 to 13] in the bone marrow group [P=0.002]), whereas bone marrow may reduce the risk of chronic GVHD at 2 years (peripheral-blood group was 53%

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[95% CI, 45 to 61], as compared with 41% [95% CI, 34 to 48] in the bone marrow group [$P=0.01$].¹⁷ The proportion of patients with extensive chronic GVHD was higher in the peripheral-blood group than in the bone marrow group (48% [95% CI, 42 to 54] vs. 32% [95% CI, 26 to 38], $P<0.001$). Among patients who were alive at 2 years, 57% of the patients in the peripheral-blood group were receiving immunosuppressive therapy, as compared with 37% of those in the bone marrow group ($P=0.03$). There were no significant between-group differences in the incidence of acute GVHD or relapse¹⁷.

Rationale for tacrolimus and Mycophenolate mofetil as immunosuppression for 180 days

With the advent of posttransplantation high-dose Cy, nonmyeloablative allogeneic HSCT platforms have been associated with acceptable rates of acute GVHD, graft failure, and nonrelapse mortality (NRM) that are similar to those seen with HLA-matched transplants.^{23,24} However, relapse remains a major problem, and approaches that augment the anti-tumor efficacy of the transplant procedure are needed. Transplantation platforms that minimize the amount of pharmacologic immunosuppression, but that carry acceptable rates of severe GVHD and graft failure, are desirable for a number of reasons.

Extensive published data demonstrate that allogeneic HSCT can be associated with a clinically significant graft-versus-tumor (GVT) effect mediated by donor T cells specific for host histocompatibility antigens. Yet, the T cells that mediate a GVT effect may also cause clinically significant GVHD. Tacrolimus, a calcineurin inhibitor (CNI), blocks T cell activation and the production of interleukin-2, a critical growth factor for T cells including regulatory T cells that control autoimmunity. CNI's are used to prevent acute GVHD, but they are associated with an increased incidence of renal dysfunction, hypertension, opportunistic infection, and other complications. Sirolimus is similarly used and applied. Importantly, CNI's block T cell development in the thymus^{16,17} resulting in delayed immunologic reconstitution, and by suppressing T cell activation may block the GVT effect and increase the risk of disease relapse after allogeneic HSCT.¹⁸⁻²⁰

MMF plus a CNI (i.e. tacrolimus or cyclosporine or sirolimus) represents standard GVHD prophylaxis for nonmyeloablative conditioning. In nonmyeloablative, HLA-matched HSCT with postgrafting immunosuppression consisting solely of MMF + cyclosporine, investigators at the Fred Hutchinson retrospectively evaluated three durations of cyclosporine: taper from Days 35 to 56, Days 56 to 77, or Days 56 to 180.³⁶ Grafts were derived from peripheral blood stem cells. There was no significant association between cyclosporine duration and the rates of acute grade II-IV GVHD (57%, 43%, and 49% respectively), extensive chronic GVHD, or NRM; however, longer duration of cyclosporine was associated with a lower risk of acute severe (grade III-IV) GVHD and lower incidence of discontinuation of all systemic immunosuppression by 24 months (an indirect marker of the prevention and successful treatment of GVHD).

In an older randomized trial of myeloablative, HLA-identical or one-antigen mismatched HSCT, where postgrafting immunosuppression consisted of methotrexate and cyclosporine with or without methylprednisolone, results suggested that cyclosporine could be stopped earlier (by Day 60) in patients without prior acute GVHD, whereas those with prior acute GVHD appeared to benefit from a longer course³⁷. In another randomized trial of myeloablative, HLA-matched related or unrelated donor HSCT, the risk of clinically extensive chronic GVHD and transplant-related mortality did not significantly differ in patients assigned to 6 months versus 24 months of cyclosporine.³⁸ Other nonrandomized studies of myeloablative, HLA-matched sibling transplant have suggested a benefit to longer duration cyclosporine in chronic GVHD prevention, however.³⁹ Some studies also have found an increased risk of relapse associated with higher doses of cyclosporine.^{40,41}

It is important to note that these experiences with immunosuppression duration with other allogeneic HSCT platforms cannot be directly extrapolated to the high-dose posttransplantation Cy platform that this protocol studies. Immunosuppression must be sufficient to prevent graft failure and to prevent excessive rates of GVHD including severe GVHD; yet extended-course immunosuppression may increase the risk of infection, drug toxicity, and relapse. There are presently no published data on the minimum required duration of tacrolimus or other immunosuppression after nonmyeloablative HSCT that includes high-dose Cy as part of postgrafting immunosuppression. The effectiveness of high-dose posttransplantation Cy in GVHD prevention, however, permits the investigation of this question.

High-dose posttransplantation cyclophosphamide

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The immunologic rationale for administering high-dose Cy after transplantation is that recently activated, alloreactive T cells (the cells most responsible for GVHD) are selectively sensitive to the toxic effects of this drug.²⁵ High-dose Cy, when administered in a narrow window after transplantation, depletes alloreactive T cells from the donor and host and can inhibit both GVHD and graft rejection.²⁵⁻³⁰ As a form of drug-induced immunologic tolerance,³¹ the strategy of giving high-dose Cy after transplantation takes advantage of the heightened cytotoxic sensitivity of proliferating, alloreactive T cells over non-alloreactive, resting T cells to being killed by a DNA-damaging agent.³² Pre-clinical studies demonstrated that engraftment of major histocompatibility complex (MHC)-mismatched bone marrow could be achieved by conditioning mice with pretransplantation fludarabine and low dose (200 cGy) total body irradiation (TBI), with posttransplantation Cy.²⁷ Additional studies demonstrated that posttransplantation Cy reduced the incidence and severity of GVHD in the setting of MHC-mismatched allogeneic HSCT after myeloablative conditioning.²⁶

After allogeneic HSCT, standard regimens of GVHD prophylaxis consist of a CNI (cyclosporine or tacrolimus) in combination with either methotrexate, MMF, or sirolimus. However, a nonmyeloablative, partially HLA-mismatched (haploidentical), related donor HSCT platform with high-dose posttransplantation Cy, MMF, and tacrolimus for GVHD and graft rejection prophylaxis has produced encouraging results²⁴. This approach has been associated with rapid and stable engraftment in most patients.²⁴ Most importantly, this approach has carried acceptable rates of GVHD and NRM that parallel those seen with nonmyeloablative HLA-matched transplants (Figure 1).^{23,24,33,34} Cy, when administered at high doses after myeloablative, HLA-matched, related or unrelated donor HSCT, notably has been found to be effective single-agent prophylaxis against GVHD, obviating the need for CNI's in this setting (Figure 1).³⁵

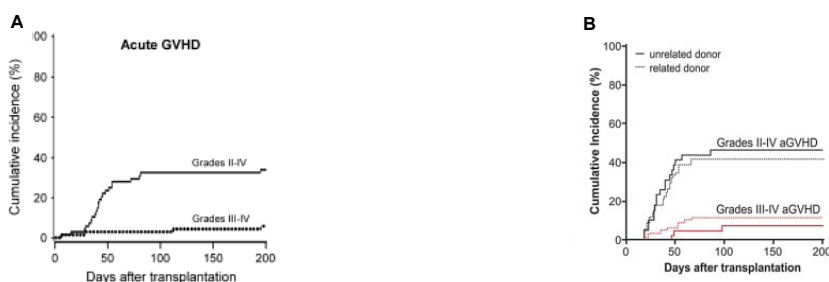


Figure 1. Acute GVHD after HSCT incorporating high-dose posttransplantation Cy. A) nonmyeloablative HSCT.²⁴ B) myeloablative, HLA-matched, related or unrelated donor HSCT, with Cy as single-agent prophylaxis.³⁵

Nonmyeloablative HSCT with fludarabine, TBI, and posttransplantation cyclophosphamide

The same approach to nonmyeloablative HSCT using partially HLA-mismatched, related donors has been adopted at Johns Hopkins for HLA-identical donor HSCT. In a phase I trial (J0169) of nonmyeloablative, matched sibling donor HSCT, Cy alone on Day 3, Cy alone on Days 3 and 4, or Cy on Days 3 and 4 with MMF was investigated with the latter being favored. Although the optimal postgrafting immunosuppression after high-dose Cy is not defined, MMF plus a CNI is standard GVHD prophylaxis for nonmyeloablative conditioning (i.e. omission of a CNI is nonstandard).

Independent clinical trials have evaluated or are evaluating a nonmyeloablative, partially HLA-mismatched (haploidentical), related donor marrow transplant platform with high-dose posttransplantation Cy, tacrolimus, and MMF for GVHD and graft rejection prophylaxis. Conditioning in these studies has historically consisted of fludarabine, low-dose Cy, and 200 cGy TBI. The postgrafting immunosuppression regimen that underlies recent and ongoing research efforts at Johns Hopkins has been published.^{24,34} A combined analysis of two independent clinical trials for poor-risk hematologic malignancies was originally reported in 2008 (40 patients at Johns Hopkins, 28 at Fred Hutchinson Cancer Research Center), evaluating the safety and efficacy of a high-dose posttransplantation Cy platform after outpatient nonmyeloablative conditioning and T-cell-replete

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HSCT from partially HLA-mismatched, related donors (Figure 2).²⁴ Following transplantation, high-dose (50 mg/kg) Cy was administered on Day 3 (Seattle group), or on Days 3 and 4 (Hopkins). Pharmacologic prophylaxis of GVHD was initiated on the day following completion of posttransplantation Cy with MMF until Day 35, and tacrolimus which was tapered to off by Day 180 (Seattle) or continued at full dose until Day 180 (Hopkins). Filgrastim 5 µg/kg/day was administered until recovery of neutrophils to >1000/µL:

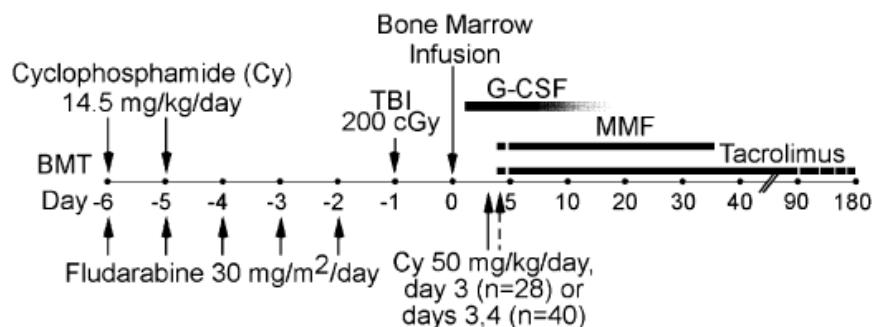


Figure 2. Treatment Schema in Previous Studies

Median times to recovery of neutrophils and platelets were 15 and 24 days, respectively. Graft failure occurred in 9 of 66 evaluable patients (12%); all but one patient with graft failure had recovery of autologous hematopoiesis with median times to neutrophil and platelet recovery of 15 days (range, 11-42) and 28 days (range, 0 – 395 days) respectively. Engrafting patients achieved full donor chimerism rapidly; with few exceptions, donor chimerism in patients with sustained engraftment was virtually complete ($\geq 95\%$) by 2 months after transplantation. The cumulative incidences of acute grade II-IV GVHD and acute grade III-IV GVHD by Day 200 were <35% and <10%, respectively, on competing-risk analysis. The groups did not differ significantly in the incidence of acute grade II-IV or III-IV GVHD, although the risk of chronic GVHD appeared to be lower with two doses of Cy. The cumulative incidence of extensive chronic GVHD by 1 year was only 5% in the group with two doses of Cy. The cumulative incidences of relapse and NRM at 1 year were 51% and 15% respectively on competing-risk analysis; however the event-free survival (EFS) probability at 1 year was only 34%. Similar outcomes were seen in a recent analysis of 185 patients treated on these trials including follow-up phase II trial (J0457) of this approach.³⁴ Interestingly, although increasing degrees of HLA mismatch between donor and recipient have historically been associated with greater GVHD and inferior survival after allogeneic HSCT, that analysis retrospectively found no such adverse effect of HLA mismatching using this approach in the haploidentical setting.³⁴ On an updated analysis of 212 patients with advanced hematologic malignancies, uniformly treated at Johns Hopkins with related donor, partially HLA-mismatched HSCT with fludarabine/Cy/TBI conditioning and postgrafting immunosuppression with 2 doses of Cy, MMF on Days 5-35, and full-dose tacrolimus on Days 5-180, the 1-year EFS was 44%, and cumulative incidence of NRM by competing risk analysis was 8% by Day 100 and 14% at 1 year. The cumulative incidence of acute grade II-IV GVHD was 28% by Day 200, cumulative incidence of severe GVHD was only 4%, and the cumulative incidence of chronic GVHD was 14% by competing risk analysis (unpublished data).

Summary

In summary, post-transplant Cy after non-myeloablative haploidentical or matched donors appears to be a generally well-tolerated procedure. The toxicity of the procedure compares favorably to the toxicity of non-myeloablative transplantation using unrelated or even HLA-identical sibling donors.²³ The post-transplant immunosuppression, which may play a role in the rate of relapse (which can be up to 50% at one year) is likely due to GVH, which in turn is related to stem cell source and immunosuppression. We propose that a nonmyeloablative conditioned HLA identical or haploidentical PBSC graft using post-transplant high dose cyclophosphamide, tacrolimus, and MMF will lead to successful engraftment, tolerable acute and chronic graft versus, potential progression free and overall survival benefit, especially in those hematologic malignancies that have been a challenge to engraft.

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1.2 Investigational Agent

Cyclophosphamide (Cytoxan®)

Cyclophosphamide is an alkylating agent whose metabolites form cross-links with DNA resulting in cell cycle-nonspecific inhibition of DNA synthesis and function.

1.3 Dose Rationale

The rationale for dosing of the preparative regimen and the post-transplant cyclophosphamide is based on the literature and described in detail in the background sections “High-dose posttransplantation cyclophosphamide”, “Nonmyeloablative HSCT with fludarabine, TBI, and posttransplantation cyclophosphamide”. The dose rationale for pos-transplant tacrolimus is also included in “Rationale for tacrolimus as immunosuppression for 180 days”.

1.4 Research Risks & Benefits

1.4.1 Risk of Study Drug

Cyclophosphamide

Very Common (> 30%chance this will happen)

- Neutropenia, Anemia and thrombocytopenia
- Alopecia
- Nausea and vomiting
- Sterility

Likely (10% to 30% chance this will happen)

- Diarrhea
- Mouth sores
- Hemorrhagic cystitis
- Fluid weight/gain
- SIADH
- Transaminitis
- Cardiomyopathy
- Pericarditis
- Rash
- Mucositis

Rare (< 10% chance this will happen)

- Secondary myelodysplastic syndrome
- Anaphylaxis

1.4.2 Other Risks of Study Participation

Allogeneic HSCT carries risk for major morbidity and mortality. Major toxicities and risks of the transplant procedure include acute and chronic GVHD, severe infection, immunosuppression which may be prolonged, graft failure, end-organ damage, and death. High-dose posttransplantation Cy appears to significantly lower the risk of GVHD. Shorter-duration immunosuppression may be associated with increased risk of GVHD, increased severity of GVHD, and graft failure. Additional risks to study participation include breach of confidentiality and risks associated with blood draw. Privacy procedures in place and good clinical practice guidelines are followed for this study to minimize risks associated with research procedures and participation. The risks associated with blood draw include weaknesses, redness, pain, bruising, bleeding or infection at the needle site.

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1.4.3 Potential benefits

The potential benefits of this trial are palliation of disease-related symptoms and prolongation of overall or event-free survival, including the possibility of long-term disease-free survival and cure. Potential benefits also include fewer infectious and other complications and lower risk of relapse because of the shorter duration of immunosuppression.

1.4.4 Cyclophosphamide FDA IND Exemption

Cyclophosphamide is currently marketed in the United States. The proposed investigation is not attended to support a new indication, significant change in the labeling, or in the advertising of this medication. The use of cyclophosphamide is not being studied in a different route, dose level, or in a different patient population that has not been studied by other investigators and has been published. The current investigation will be conducted in compliance with requirements of the IRB review as set for in 21 CFR Part 56 and with the requirements for informed consent as set forth in the 21 CFR part 50. In addition, this investigation will be conducted in compliance with 21 CFR 312.7 regarding the promotion and charging for investigational drugs.

2 Study Objectives

Overall objective

Evaluate the safety and feasibility in nonmyeloablative, partially HLA-mismatched or HLA-matched PBSC transplant from haploidentical donors or fully matched donors with post-grafting immunosuppression that includes high-dose cyclophosphamide, tacrolimus, and MMF.

Primary Objective

Estimate event free survival (EFS) (relapse, progression, or death) rate one year after transplant.

Secondary Objectives:

1. Estimate the cumulative incidences of severe acute grade III or higher GVHD, chronic GVHD (overall and by extent)
2. Estimate the cumulative incidence of systemic steroid initiation,
3. Summarize the graft failure frequency,
4. Summarize the kinetics of neutrophil and platelet recovery, and kinetics of donor chimerism in unsorted and CD3+ sorted peripheral blood.
5. Summarize major toxicities and complications associated with the transplantation procedure selected toxicities.

Exploratory Objectives:

Explore the association between the amount of donor T cell chimerism at ~ Day 28 and patient/graft characteristics (e.g., prior therapies, graft cell dose) and transplantation outcomes (sustained engraftment, relapse or progression, GVHD).

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3 Study Design

3.1 General Design

This is an open label phase II single arm study of peripheral blood stem cell transplantation and posttransplantation cyclophosphamide, using HLA full match or haploidentical related donors, in hematological malignancies including those difficult to engraft.

4 Subject Selection and Withdrawal

4.1 Selection of Patients and Donors

Eligibility for transplantation

The following are eligibility for study entry and transplantation. Eligibility criteria for protocol-driven, early cessation of immunosuppression are designated in Section 5.3.9.3.

1. Presence of a suitable related, HLA-haploidentical or HLA-matched stem cell donor
 - a. The donor and recipient must be identical at least one allele of each of the following genetic loci: HLA-A, HLA-B, HLA-Cw, HLA-DRB1, and HLA-DQB1. A minimum match of 5/10 is therefore required for related donors, and will be considered sufficient evidence that the donor and recipient share one HLA haplotype.
2. Eligible diagnoses:
 - a. Myelodysplastic syndrome (MDS) including chronic myelomonocytic leukemia [CMML] with at least one of the following poor-risk features:
 - i. Poor-risk cytogenetics
 - ii. IPSS score of INT-2 or greater
 - iii. Treatment-related or secondary MDS
 - iv. MDS diagnosed before age 21
 - v. Progression on or lack of response to standard DNA-methyltransferase inhibitor therapy
 - vi. Life-threatening cytopenias, including those requiring frequent transfusions
 - b. SLL or CLL with 17p deletion, or with progression < 6 months after second or greater treatment regimen. Must have the following to be an acceptable candidate as well:
 - i. $\leq 20\%$ of bone marrow cellularity involved by SLL/CLL (to lower risk of graft rejection)
 - ii. No lymph nodes ≥ 5 cm in any dimension
 - iii. No massive splenomegaly, defined as > 6 cm below the left costal margin
 - c. T-cell PLL in PR or better prior to transplantation. Must also have $\leq 20\%$ of bone marrow cellularity involved by PLL (to lower risk of graft rejection).
 - d. Interferon- or tyrosine kinase-refractory CML in first chronic phase, TKI-intolerant CML in first chronic phase, or CML in second or subsequent chronic phase
 - e. Philadelphia chromosome negative myeloproliferative disease (including myelofibrosis)
 - i. Intermediate-2 or High risk score by DIPSS Plus is required for a diagnosis of myelofibrosis
 - f. Multiple myeloma or plasma cell leukemia with a PR or better to the last treatment regimen, based on the International Myeloma Working Group (IMWG) criteria.⁴⁹
 - g. Hematologic malignancy in complete remission with minimal residual disease (MRD) non detectable OR detectable by conventional cytogenetics, FISH, flow cytometry, or molecular testing or hematologic malignancies in partial remission
3. No active extramedullary leukemia or known active CNS involvement by malignancy. Such disease treated into remission is permitted.
4. Any previous autologous HSCT must have occurred at least 3 months prior to start of conditioning
5. No previous allogeneic HSCT
6. Adequate end-organ function as measured by:

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- a. Left ventricular ejection fraction $\geq 35\%$ or shortening fraction $> 25\%$
- b. Bilirubin ≤ 3.0 mg/dL (unless due to Gilbert's syndrome or hemolysis), and ALT and AST $\leq 5 \times$ ULN
- c. FEV₁ and FVC $\geq 40\%$ of predicted; or if unable to perform pulmonary function tests due to young age, oxygen saturation $>92\%$ on room air
- 7. ECOG performance status ≤ 2 or Karnofsky or Lansky score ≥ 60 .
- 8. Age ≥ 18 years and older.
- 9. Not pregnant or breast-feeding.
- 10. No uncontrolled infection.

Note: Infection is permitted if there is evidence of response to medication. Eligibility of HIV infected patients will be determined on a case-by-case basis.

Donor eligibility

- 1. Donors must be either:
 - a. HLA-haploidentical or HLA-identical relatives of the patient based on allele or allele group level typing as defined in Section 4.1.
- 2. Medically fit to and willing to donate
- 3. Lack of recipient anti-donor HLA antibody

Note: In some instances, low level, non-cytotoxic HLA specific antibodies may be permissible if they are found to be at a level well below that detectable by flow cytometry. This will be decided on a case-by-case basis by the PI and one of the immunogenetics directors. Pheresis to reduce anti-HLA antibodies is permissible; however eligibility to proceed with the transplant regimen would be contingent upon the success of the desensitization.
- 4. Has not donated blood products to patient

Donor prioritization

Donors will be prioritized in the following order:

- 1. Fit to donate
- 2. HLA-matched prioritized over HLA-mismatched
- 3. Lack of major ABO incompatibility

In order of priority:

 - a. Compatible
 - b. Minor incompatibility
 - c. Major incompatibility
- 4. CMV serostatus: CMV negative donor preferred, if the patient is CMV negative; CMV positive donor preferred, if the patient is CMV positive.
- 5. Avoidance of female donor for male recipient

Other factors such as donor age and health history will be integrated into the donor selection process per standard practice and may be prioritized over HLA, ABO and CMV status.

4.2 Exclusion Criteria

Any individual that does not meet the eligibility criteria for transplantation or donor eligibility will not be a part of this trial.

4.3 Inclusion of Women and Minorities

Every effort will be made to recruit women of all races and ethnic groups to this study.

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4.4 Subject Recruitment

The eligibility criteria in this trial should not have a negative effect on the enrollment of the desired populations. Target enrollment for this trial is 15 patients within 18 months. Patients will be recruited from physicians at the NYU Langone Perlmutter Cancer Center. Consenting, screening, and treatment will take place at the NYULMC PCC under the supervision of the Principal Investigator. Prospective subjects will receive detailed information regarding this trial; its investigational nature, required study procedures, alternative treatments, risks and potential benefits of the study. They will also receive the informed consent document to read. All questions are answered by the PI and/or qualified research personnel.

The Principal Investigator will:

1. Obtain signed and dated informed consent from the potential subject before any study specific procedures are performed.
2. Determine patient eligibility See Sections 4.1 and 4.2
3. Submit registration to NYU Langone Perlmutter Cancer Center CTO
4. Receive registration confirmation form the Research Coordinator at NYU Perlmutter Cancer Center CTO, including a unique study identification number assigned to the patient by the research coordinator, which will be distributed to the study team upon registration of the patient.

Recruitment and consenting will take place in a private area such as an exam room to protect the patient's privacy. The informed consent process and documentation follows the established procedures of the NYULMC Perlmutter Cancer Center Clinical Trials Office.

4.4.1 Informed Consent

Consent will be obtained only by a participating investigator who has completed requisite training for human subject research and has been instructed by the Principal Investigator about the patients and address any questions or concerns prior to obtaining written informed consent for participation and HIPAA authorization.

Patients will be given adequate time to read the consent form. They will be given time to ask questions about the study in private exam rooms. Questions will be answered by a participating physician, or qualified research study team member all of whom have completed requisite training for human subject research. Investigators will review the informed consent form with patients and address any questions or concerns prior to obtaining written informed consent for participation. Investigators will stress that participation in the study is completely voluntary and will not affect the care patients receive or result in any loss of benefits to which patients are otherwise entitled.

The Investigator will explain to each subject the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved, potential compensation and or costs incurred by the patient and any discomfort this trial may entail. This informed consent should be given by means of standard written statement, written in non-technical language. No patient can enter the study before his/her informed consent has been obtained. All patients will be required to sign a written informed consent prior to being registered on this study. Every effort will be made to answer questions raised by patients and their families or advocates regarding the protocol and alternative therapies prior to asking a patient to sign the consent form.

The informed consent form is considered to be part of the protocol, and must be submitted by the investigator with it for IRB approval.

If applicable

For non-English speaking patients, institutional translation services will be utilized. All procedures for consenting non-English speaking patients will be accordance with NYU Langone Health PCC CTO guidelines and policies.

For patients who cannot read. A witness, not related to the research study will be present. The consent will be read to the patient. The patient will also be allowed to ask any questions s/he may have. The investigator will ask the patient questions to ensure s/he understands the study. If the investigator determines the subject

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understands the study, the patient will mark an X where his/her name would go and the witness will sign the consent form.

4.4.2 Documentation of Consent

The Principal Investigator or IRB approved sub-investigator will be responsible for documentation in the medical record that consent has been obtained from all participants. A signed copy of the consent form will be given to each participant. Original consent forms and informed consent checklists will be stored in the subject's medical chart.

4.5 Registration Procedures

4.5.1 General Guidelines

Each patient must sign and date an informed consent form before undergoing any study specific procedure unless a procedure is being performed as part of the patient's standard of care.

Enrollment in the study requires that all inclusion and exclusion criteria have been met. Enrollment occurs upon confirmation of registration from the NYULMC PCC Clinical Trials Office. The following materials must be submitted to the Research Coordinator for subject registration:

1. Complete signed and dated informed consent form
2. Complete signed and dated eligibility checklist
3. All supporting documentation verifying each criterion has been met

Registration will occur within 24 hours of research coordinator receipt of all of the above documents. A written confirmation of enrollment including a unique study identification number assigned by the research coordinator will be disbursed to the study team upon registration.

Once eligibility is verified, a unique patient study number will be issued within 24 hours of receiving all required registration material. The patient will not be identified by name. This is the point, at which, the patient is considered on study. Subjects must not start any protocol study specific procedures, unless part of standard of care prior to registration.

4.6 Early Withdrawal of Subjects

4.6.1 When and How to Withdraw Subjects

A subject has the right to voluntarily discontinue study treatment or withdraw from the study at any time, for any reason, and without repercussion. The investigator also has the right to discontinue a patient from study treatment or to withdraw a patient from the study at any time.

Reasons for subject withdrawal from the study may include, but are not limited to:

- Subject withdrawal of consent at any time.
- Disease progression
- Intolerable toxicity
- Any medical condition that the investigator determines may jeopardize the patient's safety if s/he continues in the study or continues treatment with study drug.
- The investigator determines it is in the best interest of the patient.
- Failure of the subject to adhere to protocol procedure requirements
- Pregnancy

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- Failure to initiate conditioning regimen

The reason for withdrawal and the date of participant withdrawal must be documented in the case report form (CRF).

4.6.2 Data Collection and Follow-up for Withdrawn Subjects

If subjects are withdrawn prematurely from the study all attempts will be made to continue to follow-up and collect survival data and other data as outlined in the outcomes section. Patients will be called a minimum of three times a week for three weeks at all numbers available in the demographic section of their charts, then next of kin, and then certified letter will be sent.

5 Study Drug

5.1 Description

Cyclophosphamide is an alkylating agent whose metabolites form cross-links with DNA resulting in cell cycle-nonspecific inhibition of DNA synthesis and function. Dose adjustments for cyclophosphamide will not be made.

5.2 Trial Treatment

The treatment to be used in this trial is outlined below in Table 1

Table 1 Trial Treatment

Drug	Dose/Potency	Dose Frequency	Route of Administration	Regimen/Treatment Period
Fludarabine	30mg/m ²	Daily	IV infusion	Day -6, -5
Cyclophosphamide	14.5 mg/kg	Daily	IV infusion	Day -6, -5
Cyclophosphamide	50 mg/kg	QD	IV infusion	Day 3, 4
Mesna	40 mg/kg	QD	IV infusion	Day 3, 4
Mycophenolate Mofetil	15mg/kg	TID	PO	Day 5
Tacrolimus	2 mg	BID	PO	Day 5
Sirolimus ^a	1mg	BID	PO	Day 5

^aSirolimus will be administered for patients that must switch from Tacrolimus, due to clinical reasons(e.g. tacrolimus nephrotoxicity or PRES)

5.3 Treatment Regimen

5.3.1 Transplantation regimen

The preparative regimen in each case consists of fludarabine, Cy, and TBI, with posttransplantation high-dose Cy, MMF, and tacrolimus. Tacrolimus may be replaced by sirolimus for clinical reasons (e.g. nephrotoxicity or PRES).

5.3.2 Fludarabine

Fludarabine 30 mg/m²/day (adjusted for renal function) is administered over a 30-60 minute IV infusion on Days –6 through –2 (maximum cumulative dose, 150 mg/m²).

The body surface area (BSA) for fludarabine dosing is based on actual body weight.

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For decreased creatinine clearance (CrCl), fludarabine dosage is reduced as follows or by institutional standard:

CrCl 40-69 mL/min, fludarabine = 24 mg/m²

CrCl 20-39 mL/min, fludarabine = 20 mg/m²

For patients \geq 18 years old, CrCl will be estimated by the Cockcroft Formula, based on ideal body weight (IBW):

$$\text{CrCl} = \frac{(140 - \text{age}) \times \text{IBW (kg)}}{P_{\text{Cr}} \times 72} \times 0.85 \text{ for females}$$

A measured CrCl or a glomerular filtration rate may be substituted to determine CrCl.

Fludarabine dosing is based on the last CrCl prior to the start of conditioning. The estimated CrCl on the day preceding start of conditioning may be used. The fludarabine dose should be the same on Days -6 to -2, even if the creatinine changes. However, adjustment in fludarabine dose due to creatinine changes during conditioning is permitted.

5.3.3 Pretransplantation cyclophosphamide

Cy 14.5 mg/kg/day is administered as a 1-2 hour IV infusion on Days -6 and -5 after hydration. Mesna 11.6 mg/kg IV daily on Days -6 and -5 is not required, but may be given.

Cy and mesna are dosed according to IBW, unless the patient weighs less than IBW, in which case dose drug according to actual weight.

5.3.4 Total body irradiation

200 cGy TBI is administered in a single fraction on Day -1. Radiation sources, dose rates, and shielding follow institutional practice.

5.3.5 Day of rest

A day of rest, i.e. after preparative regimen completion and prior to peripheral blood stem cell infusion, is not routinely scheduled. Up to two days of rest may be added in this window based on logistical considerations or clinically as indicated. For one day of rest, fludarabine would be administered on Days -7 through -3, pretransplantation Cy on Day -7 and Day -6, and TBI on Day -2. For two days of rest, fludarabine would be administered on Days -8 through -4, pretransplantation Cy on Day -8 and Day -7, and TBI on Day -3.

5.3.6 Peripheral blood stem cell transplantation

On Day 0, peripheral blood stem cells are infused. The target stem cell dose is between 2×10^6 /kg and 10×10^6 /kg (actual body weight) CD34⁺ cells. Sample of the product to be infused will be sent for flow cytometry to determine the content of CD34⁺ and CD3⁺ cells. Graft dose including total nucleated cells infused/kg, CD34⁺ cells infused/kg, and CD3⁺ cells infused/kg will be recorded. The maximum CD34⁺ cell dose is 10×10^6 /kg. If more than 10×10^6 /kg CD34⁺ stem cells are collected, the excess will be discarded and not administered to the patient. Up to two leukapheresis procedures may be performed to obtain the minimum CD34⁺ cell target. If, after two leukapheresis procedures, fewer than 2×10^6 /kg CD34⁺ cells have been collected, a bone marrow harvest will be recommended and the patient will be taken off trial.

The graft will not be manipulated to deplete T cells. Processing for ABO incompatibility follows institutional practices. Guidelines for peripheral blood stem cell infusion are established and outlined

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in the ABO compatible/minor mismatched allogeneic HSCT or the ABO incompatible allogeneic HSCT standing orders.

5.3.7 Posttransplantation cyclophosphamide

Hydration prior to and following Cy, management of volume status, and monitoring for hemorrhagic cystitis will follow institutional standards. Mesna will be used with posttransplantation Cy as per institutional standards.

Cy and mesna are dosed according to IBW, unless the actual body weight is less, in which case dose drugs according to actual body weight.

Cy 50 mg/kg IV, over approximately 1-2 hours (depending on volume), is given on Day 3 posttransplantation (ideally between 60 and 72 hours after marrow infusion) and on Day 4 (approximately 24 hours after Day 3 Cy).

It is crucial that no systemic immunosuppressive agents are given from Day 0 until at least 24 hours after the completion of the posttransplantation Cy. This includes corticosteroids as anti-emetics.

5.3.8 Mycophenolate mofetil

MMF begins on Day 5, at least 24 hours after completion of posttransplantation Cy. The MMF dose is 15 mg/kg PO TID (actual body weight) with total daily dose not to exceed 3 grams (i.e. maximum 1 g PO TID). Doses are rounded to the nearest strength tablets. Equivalent IV dosing (1:1 conversion) may instead be given. Guidelines for dose modification are provided in Section 8.15. MMF prophylaxis is discontinued after the last dose on Day 35, or may be continued if there is GVHD.

5.3.9 Tacrolimus (or other immunosuppression with sirolimus)

5.3.9.1 Tacrolimus initiation and dosing

Tacrolimus begins on Day 5, at least 24 hours after completion of posttransplantation Cy. Duration of tacrolimus is designated in Section 5.3.9.2.

For patients > 18 years old, the tacrolimus starting dose is 2 mg PO BID or as per institutional standard. Patients who cannot tolerate PO may be started IV and changed to PO as per institutional standard. Dose is adjusted to maintain a serum trough level of **10 – 15 ng/mL**, with a minimum acceptable trough level of 5 ng/mL.

Tacrolimus can be changed to a PO BID dosing schedule once a stable therapeutic level is achieved and the patient can tolerate PO medications. Dose is adjusted to maintain a serum trough level of **10 – 15 ng/mL**, with a minimum acceptable trough level of 5 ng/mL.

In the case of prohibitive toxicities to calcineurin inhibitors (e.g., PRES), other immunosuppression may be given after case-by-case discussion with the PI or co-PI. Sirolimus is discussed here as the alternative. There are known patients s/p BMT as well as solid organ transplant who are able to tolerate sirolimus better from the nephrotoxicity standpoint. If there are patients who have creatinine rises that the primary clinicians are not comfortable with in the setting of tacrolimus, sirolimus is a reasonable alternative. Additionally there are more reports of posterior reversible encephalopathy syndrome (PRES) with tacrolimus and this would necessitate a switch to sirolimus as well.

5.3.9.2 Duration of immunosuppression

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Immunosuppression is discontinued after the last dose on Day 180 without taper; however in these patients, immunosuppression may be continued beyond Day 180 if GVHD has occurred or may be discontinued earlier in the context of relapse, progression, graft failure, or prohibitive toxicity. Patients with suspected graft failure should remain on immunosuppression until at least the ~Day 56 chimerism assessment, although earlier discontinuation is permissible after discussion with the PI or co-PI.

5.9.3.3 Evaluability for the primary endpoint

For protocol-driven, early cessation of immunosuppression, stopping immunosuppression up to 5 days after the scheduled stop date is permissible for logistical reasons. Such patients will be considered evaluable for both the safety and feasibility of early immunosuppression cessation.

Patients who should stop immunosuppression within this time frame (per protocol-driven criteria), but do not for logistical reasons, are unevaluable for safety; whether they remain evaluable for feasibility depends on the cause. If immunosuppression is not stopped in the allowable window because of pending evaluations, feasibility will be based on the results of those evaluations, provided they were done no later than 5 days past the pre-specified date of immunosuppression cessation. Cases in which eligible patients do not stop immunosuppression early because of physician discretion will count *against* feasibility.

Patients who are not evaluable for the primary endpoint may, if needed, be replaced but will continue on study unless consent is withdrawn.

5.3.10 Growth factors

GCSF (filgrastim) begins on Day 5 at a dose of 5 mcg/kg/day (actual body weight) IV or subcutaneously (rounding to the nearest vial dose is allowed), until the absolute neutrophil count (ANC) is $\geq 1,000/\text{mm}^3$ over the course of three days. Additional GCSF may be administered as warranted. Pegfilgrastim (Neulasta®) and GM-CSF are not permitted.

5.4 Preparation and Administration of Study Drug

Although cyclophosphamide will be administered for a non-standard indication (post-transplant immunosuppression), this agent is one of the most widely used agents in hematological malignancies and in transplant specifically. Cyclophosphamide will be prepared and supplied as per standard NYULMC policy.

5.4.1 Cyclophosphamide

Cyclophosphamide 14.5 mg/kg/day will be administered on Days -6 and -5 after hydration. It will be administered as a 1-2 hour IV infusion.

Cyclophosphamide 50 mg/kg IV, over approximately 1-2 hours (depending on volume), will be given on Day 3 posttransplantation (ideally between 60 and 72 hours after marrow infusion) and on Day 4 (approximately 24 hours after Day 3 Cy).

5.4.2 Fludarabine, mesna, mycophenolate mofetil, Tacrolimus or Sirolimus

Fludarabine (30 mg/m²/day) will be administered Days -6 through -2, with a maximum cumulative dose, 150 mg/m²

Mesna (11.6 mg/kg) may be given on Days -6 and -5, after cyclophosphamide administration.

Mycophenolate mofetil (15 mg/kg) will be begin on Day 5, with total daily dose not exceeding 3 grams.

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Tacrolimus (2mg) or Sirolimus (2mg) begins on Day 5, with a maintenance serum trough level of 10-15 ng/ml.

Criteria for treatment and dose modifications for each of these drugs will occur as per institutional standards of care.

5.5 Subject Compliance Monitoring

Subjects who are significantly non-compliant with protocol required visits, assessments, and/or dosing instructions may be withdrawn from the study by the investigator. The investigator has the right to discontinue a subject from study treatment or withdraw a subject from the study at any time.

Participants will be removed from the study when any of the following criteria apply:

- Lost to follow-up
- Withdrawal of consent for data submission
- Death

The reason for taking a participant off study, and the date the participant was removed, must be documented in the case report form (CRF).

5.6 Prior and Concomitant Therapy

Patients will be able to maintain prior medications, or be placed upon medications (including post-transplant maintenance therapy) according to institutional guidelines (e.g. vaccination) or upon the discretion of the Transplant Attending responsible for the patient's care.

5.7 Supportive care

Patients will receive transfusions, nutritional support, infection prophylaxis and treatment, and other supportive care according to standard of care and institutional guidelines.

5.7.1 Anti-ovulatory treatment

Menstruating females should begin an anti-ovulatory agent before starting the preparative regimen.

5.7.2 Intravenous access

A central venous catheter is required for administration of IV medications and blood products.

5.7.3 Infection prophylaxis

Patients will receive infection prophylaxis and treatment according to NYULMC institutional guidelines. Infection prophylaxis should include agents or strategies to prevent herpes simplex, CMV, *Pneumocystis jirovecii*, fungal infections, and infections from oral flora secondary to mucositis.

Posttransplantation immunizations will be given per institutional standard.

5.7.4 Antiemetics

Note that steroids should not be used as an antiemetic agent after the graft is infused, until at least 24 hours after the completion of all posttransplantation Cy. The use of steroids as antiemetics after this time frame is discouraged in the absence of relapsed/progressive disease.

5.8 Posttransplantation therapies

5.8.1 Donor lymphocyte infusion (DLI)

Prophylactic posttransplantation DLI (e.g., for persistent detectable malignancy, prophylaxis in the absence of detectable malignancy, or mixed donor chimerism) is not permitted before Day 200, as this carries a high risk of GVHD. The use of DLI will be recorded and such patients will be censored

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for analysis of disease and graft failure outcomes, GVHD, and related transplant-related toxicity outcomes. Analysis of outcomes without such censoring is also planned.

5.8.2 Posttransplantation systemic therapy

Preemptive systemic chemotherapy or biologic therapy (e.g., hypomethylating agent for MDS, tyrosine kinase inhibitor for Philadelphia chromosome-positive malignancy) is permitted after transplantation. Intrathecal chemotherapy is permitted.

5.8.3 Posttransplantation radiation

Consolidative radiation therapy is permitted after transplantation.

The use of preemptive therapy will be recorded. Patients who receive such therapies will not be censored for analysis of disease outcomes at that time, except as stated in Section 5.8.1.

5.9 *Posttransplantation follow-up*

Required evaluations are designated in Section 7.0.

More frequent monitoring of disease status, vital status, and toxicities may be performed for study purposes including through collection of outside records and patient and physician contact. Patients who relapse or progress will continue to be followed on study unless consent is withdrawn.

Patients will be followed primarily at NYULMC at least until the ~ Day 56 evaluations, then periodically thereafter as designated in Section 7.0. In the event that it is not possible or practical for a patient to come back to NYULMC for required evaluations, clinical and laboratory evaluations performed through a local oncologist may fulfill study requirements. This will be decided on a case-by-case basis by the PI or co-PI.

5.10 *Packaging*

Although cyclophosphamide will be administered for a non-standard indication (post-transplant immunosuppression), this agent is one of the most widely used agents in hematological malignancies and in transplant specifically. Cyclophosphamide will be supplied by the oncology pharmacy in standard packaging, and supplied as per standard NYULMC policy.

5.11 *Receiving, Storage, Dispensing and Return*

Although cyclophosphamide will be administered for a non-standard indication (post-transplant immunosuppression), this agent is one of the most widely used agents in hematological malignancies and in transplant specifically. Cyclophosphamide will be received, stored, dispensed and/or destroyed by the oncology pharmacy as per standard NYULMC policy.

6 Study Procedures

The Study Parameter-Section 7 summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.

6.1 *Standard Pre/Posttransplant evaluations*

Demographics and baseline characteristics will be captured. Characteristics to be recorded include: age, gender, race/ethnicity, performance status, disease type, remission status, prior treatments including prior transplantation and type, donor age, donor relationship to patient, donor gender, type of transplant (HLA-matched or mismatched), CMV sero status of patient and donor, ABO compatibility.

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Other data captured, according to NYULMC Standard Operating Procedures include those that are reported to the Center for International Blood and Marrow Transplant program.

6.2 Baseline Period

The baseline period occurs < 1 month before initiation of conditioning therapy. Results of evaluations performed before study entry as standard of care may be used for research purposes and to fulfill study requirements.

6.3 Treatment period

The treatment period includes D-6, when patients begin their preparative regimen, to Day 180 when immunosuppression is discontinued.

6.4 Post-treatment visits-Follow up Visits

All follow-up visits after 180 day will be up to the discretion of the Transplant Attending.

6.5 Research Specimen Procedures

All specimens obtained in this study, comprised of peripheral blood for chimerism studies and potentially skin and other biopsies to evaluate for GVH, are to be done as part of standard practice as part of the Transplant Procedure. Thus their procurement and analysis and storage will be performed by a clinical NYULMC laboratory, or a laboratory contracted by NYULMC, according to their standard protocols.

7 Study Parameters

The following table summarizes the minimum testing and clinical assessments required for study purposes. This is in addition to other testing and assessments indicated as standard of care, which may be collected for study purposes.

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Table 2: Study Parameters

	Baseline	D28 +/- 3 d	D56 +/- 3 d	D84 +/- 5 d	D112 +/- 5 d	D180 +/- 7 d	D270 +/- 21 d	D365 +/- 30 d ^c
Standard pre/posttransplant evaluations ^{a, b}								
History and physical exam	X		X	X ^l	X ^l	X ^l	X ^l	X
Performance status	X							
CBC / differential ^d	X	X	X	X ^{k,l}	X ^{k,l}	X ^{k,l}	X ^{k,l}	X ^{k,l}
Comprehensive metabolic panel (CMP) ^e	X		X	X ^k	X ^k	X ^k	X ^k	X ^k
Infectious disease evaluations ^f	X							
Serum HCG (if applicable)	X							
LV ejection fraction	X							
Pulmonary function tests	X							
Bone marrow biopsy and aspirate with flow cytometry and relevant cytogenetic and molecular studies ^g	X		X, with chimerism analysis ^h			X, with chimerism analysis ^h		X, with chimerism analysis ^h
CT, PET/CT, or MRI of chest, abdomen, and pelvis (CLL only)	X		X			X ^l		X ^l
Response assessment to last therapy ⁱ	X							
HLA typing	X							
Lymphocytotoxic antibody screen	X							
Donor marrow or blood for VNTR or RFLP analysis	X							
Patient blood for baseline VNTR or RFLP analysis	X							
Peripheral blood chimerism, both total leukocyte (unsorted) and T-cell sorted		X	X	X ^k	X ^k	X ^k	X ^k	X ^k
GVHD and other morbidity assessments ^j			X	X ^k	X ^k	X ^k	X ^k	X ^k

^a Baseline evaluations studies should occur < 1 month before initiation of conditioning therapy but can be performed within the time interval outlined in the most recent version of the NYU BMT service standard operating proceedings. HLA typing and baseline studies for chimerism determinations may occur at any point prior. Results of evaluations performed before study entry as standard of care may be used for research purposes and to fulfill study requirements.

^b Demographics and baseline characteristics will be captured. Characteristics to be recorded include: age, gender, race/ethnicity, performance status, disease type, remission status, prior treatments including prior transplantation and type, donor age, donor relationship to patient, donor gender, type of transplant (HLA-matched or mismatched), CMV serostatus of patient and donor, ABO compatibility.

^c Patients should continue to follow-up at NYULMC at least yearly on study, per institutional standard of care. Follow-up data may be captured more frequently for study purposes. Data that will continue to be recorded beyond 1 year include disease status

until first relapse/progression, vital status, major transplant-related toxicities, and GVHD.

^d At minimum, CBC/differential should also be performed twice a week from start of preparative regimen, until ANC is ≥1000/uL over course of 3 days, then weekly until 12 weeks posttransplantation, and then periodically thereafter at the Transplant Attending's discretion. All data will be captured for the duration of the trial.

^e CMP includes: BUN, creatinine, sodium, potassium, chloride, AST, ALT, total bilirubin, alkaline phosphatase. At minimum, these should be performed weekly until 12 weeks posttransplantation, then periodically until off immunosuppression; those need not be captured in the CRF.

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^f Infectious disease evaluations follow institutional standard of care. Minimum evaluations are CMV IgG, HSV IgG, VZV IgG, hepatitis

panel (Hep B surface antigen, Hep B core antibody, Hep C antibody), HIV antibody (unless known to be HIV positive).

^g Follow-up studies should include relevant cytogenetics and molecular markers to detect residual disease, i.e. repeat of studies found to be positive at baseline. At the discretion of the PI, in certain circumstances a pre-transplant bone marrow biopsy maybe deferred.

^h May be omitted if there is documented disease persistence, progression or relapse before scheduled assessment.

ⁱ Include comparison of pre- and post-treatment scans with bidimensional measurements where relevant.

^j GVHD and other morbidity assessments are also standardly performed weekly at NYULMC until at least ~Day 60. Results of these and subsequent assessments may be collected for research purposes. Patients may be asked to complete GVHD questionnaires.

^k Day 84 and later CMP, chimerism, and GVHD evaluations may be omitted in patients with documented graft failure. CBC/differential, CMP, H & P, and morbidity assessment at these time points will be obtained.

^l May be omitted in patients who receive treatment for disease persistence, progression, relapse. The dates of treatment initiation and DLI will be recorded.

8 Measurement of Effect and Endpoints

8.1 Hematologic parameters

8.1.1 Neutrophil recovery: Post-nadir ANC $\geq 500/\text{mm}^3$ for three consecutive measurements on different days. The first of the three days will be designated as the day of neutrophil recovery.

8.1.2 Platelet recovery: Sustained platelet count $\geq 20,000/\text{mm}^3$ or $\geq 50,000/\text{mm}^3$ with no platelet transfusions in the preceding seven days. The first of three consecutive measurements on different days will be designated as the day of initial platelet recovery.

8.1.3 Donor chimerism: Mixed donor chimerism is defined as $\geq 5\%$, but $< 95\%$, donor. Full donor chimerism is defined as $\geq 95\%$ donor.

Prior to transplantation, a sample of peripheral blood from the patient, and either harvested peripheral blood stem cells or blood from the donor, are collected for genetic studies to establish a baseline for subsequent chimerism assays.

Chimerism determinations from T cells (CD3⁺ sorted) and whole blood (total nucleated cells) will be made from peripheral blood per Section 7.0, and more frequently as indicated. Methods may include (i) PCR analysis of variable number of tandem repeats (VNTR) in PBMC if informative, (ii) restriction fragment length polymorphism (RFLP) if the donor and recipient RFLPs are informative, (iii) fluorescence in-situ hybridization (FISH) for Y-chromosome markers on PBMC if the donor is male and patient is female, (iv) cytogenetic analysis, (v) flow cytometric analysis of HLA-A, B or DR on lymphocytes in the peripheral blood if haploidentical and suitable reagents exist. Chimerism may also be determined from the bone marrow.

8.1.4 Graft failure: $< 5\%$ donor chimerism in blood and/or bone marrow on ~Day 28 or after and on all subsequent measurements.

- Primary graft failure: $< 5\%$ donor chimerism in blood and/or bone marrow by ~ Day 56
- Secondary graft failure: achievement of $\geq 5\%$ donor chimerism, followed by sustained $< 5\%$ donor chimerism in blood and/or bone marrow.

$< 5\%$ donor T cell chimerism, but with $\geq 5\%$ donor chimerism in total leukocytes, is not considered graft failure.

8.2 Graft-versus-host disease

8.2.1 Acute GVHD: Acute GVHD is graded by standard criteria (Appendix).⁴² All suspected cases of acute GVHD must be confirmed histologically by biopsy of an affected organ (e.g., skin, liver, or gastrointestinal tract). Date of symptom onset, date of biopsy confirmation of GVHD, maximum clinical grade, sites affected, and dates

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and types of treatment will be recorded. Dates of symptom onset of initial diagnosis of GVHD (even if non-severe) and grade III-IV GVHD will be recorded.

8.2.2 Chronic GVHD: Chronic GVHD is graded by both NIH consensus criteria⁴³ and Seattle criteria.⁴⁴ Date of onset, date of biopsy confirmation (if any), dates and types of treatment, and extent will be recorded. The cumulative incidence of chronic GVHD (overall and according to extent) will be determined through competing risk analysis.

8.3 Disease and survival endpoints

8.3.1 Event free Survival (EFS): Interval from Day 0 to date of first objective disease progression or relapse, or death from any cause. Patients without these failures will be censored at the last date they were assessed and deemed failure-free.

8.3.2 Relapse or progression:

Defined per the following response criteria:

- a. CLL: 2008 International Workshop criteria ⁴⁵
- b. MDS: 2006 IWG criteria ⁴⁶

Designation of disease status in other histologies will also follow standard criteria.

9 Statistical Methods

9.1 Primary endpoint and design

Estimate the one year after transplantation event free survival (EFS) rate using a Kaplan-Meier curve with a 90% confidence interval. An event for EFS is defined as the first of any of the following failures: relapse or disease progression or death from any cause.

9.2 Secondary endpoints

- a. Estimate the cumulative incidences of severe acute grade III or higher GVHD, chronic GVHD (overall and by extent) by one year
- b. Estimate the cumulative incidence of systemic steroid initiation, by 1 year after HSCT
- c. The above cumulative incidences (a. & b.) will be similarly estimated using competing-risk analyses, wherein graft failure and death, or graft failure, death and treatment of relapse/progression, are considered competing risks. The number and types of systemic immunosuppression used for GVHD treatment will be reported.
- d. Summarize graft failure frequency. The graft failure frequency in evaluable patients (those having chimerism results at least at ~Day 28) will be estimated with 90% confidence intervals.
- e. Summarize kinetics of neutrophil and platelet recovery, and kinetics of donor chimerism in unsorted and CD3+ sorted peripheral blood. Times to neutrophil and platelet recovery will be summarized using medians and ranges, and with cumulative incidence functions with death before count recovery as a competing risk. The degree of donor chimerism in unsorted and CD3+ sorted peripheral blood at predefined time points (per Section 7.0) will be summarized using medians and ranges, and the proportion reaching full donor chimerism (total leukocyte, T cell) by ~ Day 28 and ~ Day 56 will be estimated with 90% confidence intervals. The proportion achieving >50% T cell donor chimerism at ~ Day 28 and ~ Day 56 will similarly be estimated.
- f. Summarize major toxicities and complications associated with the transplantation procedure selected toxicities.

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9.3 Exploratory endpoints

Explore the association between the amount of donor T cell chimerism at ~ Day 28 and patient/graft characteristics (e.g., prior therapies, graft cell dose) and transplantation outcomes (sustained engraftment, relapse or progression, GVHD). Because of the limited sample size these investigations will be exploratory.

9.4 Sample Size Determination

The primary goal of this study is to estimate the one year event free survival (EFS) rate after transplantation in both HLA haploidentical and identical related donors with post-transplant cyclophosphamide and the MF and tacrolimus regimen as described. In addition, we will estimate the Grade III/IV GVHD- and grade III/IV chronic GVHD-free incidence after transplantation.

Our predicted outcomes are based on published data from other studies using either bone marrow or peripheral blood as a source for stem cells and other immunosuppression regimens. These data indicate that the cumulative incidences of acute grade II-IV GVHD and acute grade III-IV GVHD by Day 200 were ~35% and ~10%, respectively. The cumulative incidence of extensive chronic GVHD by 1 year was only 5% in the group with two doses of Cy. The event-free survival (EFS) (relapse, progression or death) probability at 1 year was only 34%. Similar outcomes were seen in a recent analysis of 185 patients treated on these trials including follow-up phase II trial of this approach.³⁴ On an updated analysis of 212 patients with advanced hematologic malignancies, uniformly treated at Johns Hopkins with related donor, partially HLA-mismatched HSCT with fludarabine/Cy/TBI conditioning and postgrafting immunosuppression with 2 doses of Cy, MMF on Days 5-35, and full-dose tacrolimus on Days 5-180, the 1-year EFS (relapse, progression or death) was 44%, The cumulative incidence of acute grade II-IV GVHD was 28% by Day 200, cumulative incidence of severe GVHD was only 4%, and the cumulative incidence of chronic GVHD was 14% by competing risk analysis (unpublished data).

Based on these data we will compare our findings to the published haploidentical transplant literature (including bone marrow as a source and a range of post-transplant immunosuppression) which demonstrate a 1 year EFS-defined as relapse, progression, or death of 45%.

Assuming a historical one-year EFS rate of 45%, accrual time of 1.5 years, and follow up of 1 years from the last patient accrued, a two-sided, one-sample log-rank test based on a sample of 15 patients achieves 80% power at a 10% significance level to detect an increase of 28% in one-year EFS rate (to a total of one-year EFS rate of 73%) under the new regimen.

9.5 Subject Population(s) for Analysis

All patients enrolled onto this study will be analyzed for the primary and secondary endpoints.

10 Expected Toxicities

10.1 Drug Information

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10.1.1 Cyclophosphamide (Cytoxan®)

Cyclophosphamide is an alkylating agent whose metabolites form cross-links with DNA resulting in cell cycle-nonspecific inhibition of DNA synthesis and function. Cyclophosphamide side effects include: nausea, vomiting, diarrhea, headache, dizziness, hemorrhagic cystitis, fluid weight gain/edema, SIADH, transaminitis, cardiomyopathy, pericarditis, rash, mucositis, alopecia, cytopenias, sterility, and rarely, secondary myelodysplastic syndrome and anaphylaxis.

Dose adjustments for cyclophosphamide will not be made.

10.1.2 Mesna (sodium-2-mercapto ethane sulphonate)

Mesna is a prophylactic agent used to prevent hemorrhagic cystitis induced by the oxasophosphorines (cyclophosphamide and ifosfamide). It has no intrinsic cytotoxicity and no antagonistic effects on chemotherapy. Mesna binds with acrolein, the urotoxic metabolite produced by the oxasophosphorines, to produce a non-toxic thioether and slows the rate of acrolein formation by combining with 4-hydroxy metabolites of oxasophosphorines.

The total daily dose of mesna is equal to 80% of the total daily dose of cyclophosphamide.

At the doses used for uroprotection, mesna is virtually non-toxic. However, potential adverse effects include nausea and vomiting, diarrhea, abdominal pain, altered taste, rash, urticaria, headache, joint or limb pain, hypotension, and fatigue.

10.1.3 Fludarabine (Fludara)

Fludarabine is a purine analog antimetabolite. Side effects of fludarabine include:

- a. Neurotoxicity: Agitation or confusion, blurred vision, loss of hearing, peripheral neuropathy or weakness have been reported. Severe neurologic effects, including blindness, coma, and death may occur; severe CNS toxicity is rarely seen with doses in the recommended range for nontransplant therapy. The dose used in this study is approximately 1.5 times the usual one-course dose given in non-transplant settings. Doses and schedules similar to those used in this study have been used in adult and pediatric patients without observed increase in neurotoxicity.
- b. Anemia: Life-threatening and sometimes fatal autoimmune hemolytic anemia has been reported after one or more cycles of therapy in patients with or without a previous history of autoimmune hemolytic anemia or a positive Coombs' test and who may or may not be in remission. Corticosteroids may or may not be effective in controlling these episodes. The majority of patients re-challenged developed a recurrence of the hemolytic process.
- c. Cardiovascular: Deep venous thrombosis, phlebitis, transient ischemic attack, and aneurysm (1%) are reported.
- d. Fever: 60% develop fever.
- e. Rash: 15% develop a rash, which may be pruritic.
- f. Digestive: Gastrointestinal side effects include: nausea/vomiting (36%), diarrhea (15%), stomatitis (9%), anorexia (7%), GI bleeding and esophagitis (3%), mucositis (2%), liver failure, abnormal liver function test, constipation, dysphagia (1%) and mouth sores.
- g. Some other effects include: Chills (11%), peripheral edema (8%), myalgias (4%), osteoporosis (2%), pancytopenia, arthralgias (1%), dysuria (4%), urinary tract infection and hematuria (2%); renal failure, abnormal renal function test, and proteinuria (1%); and, very rarely, hemorrhagic cystitis and pulmonary toxicity.

Dose adjustments of fludarabine are required for renal insufficiency (see Section 5.21).

10.1.4 Total Body Irradiation (TBI)

TBI can cause: nausea and vomiting, diarrhea, parotitis (rapid onset within 24-48 hours, usually self-limited),

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generalized mild erythema (usually within 24 hours, resolving in 48-72 hours), hyperpigmentation, fever, mucositis, alopecia, and pancytopenia. Late effects include: cataracts (10-20%), hypothyroidism, nephropathy, interstitial pneumonitis, veno-occlusive disease, carcinogenesis, and sterility.

10.1.5 **Mycophenolate Mofetil (MMF, Cellcept®)**

MMF is an ester prodrug of the active immunosuppressant mycophenolic acid (MPA).

Side effects include: pancytopenia, infection (including sepsis, CMV, HSV, VZV, and Candida), nausea, vomiting, diarrhea, allergic reactions, hypertension, headache, dizziness, insomnia, hyperglycemia, electrolyte imbalances, rash, and leg cramps/bone pain.

Drug interactions: MMF activity is decreased with oral antacids and cholestyramine. There are no pharmacokinetic interactions with cotrimoxazole, oral contraceptives, or cyclosporine. Acyclovir or ganciclovir blood levels may increase due to competition for tubular secretion. High doses of salicylates or other highly protein-bound drugs may increase the free fraction of MPA and exaggerate the potential for myelosuppression.

Dose adjustments: No dose adjustments are required for liver dysfunction. For renal insufficiency, MMF dosing should not be modified unless dialysis is needed, in which case MMF can be reduced to 25-50% of the starting dose.

10.1.6 **Tacrolimus (FK-506, Prograf®)**

Tacrolimus is a macrolide immunosuppressant that inhibits lymphocytes through calcineurin inhibition.

Toxicities: There is a spectrum of well-described toxicities of tacrolimus. Toxicities include renal insufficiency, hypertension, hyperglycemia, hypomagnesemia, hypokalemia, nausea, diarrhea, headache, neurologic toxicity including tremor and leukoencephalopathy, infection, and rarely thrombotic thrombocytopenic purpura (TTP).

Drug interactions: Tacrolimus is well absorbed orally. Tacrolimus is extensively metabolized by the cytochrome P-450 (CYP3A4) system and metabolized products are excreted in the urine. Drugs that may increase tacrolimus levels include tri-azole drugs (especially voriconazole and posaconazole), nephrotoxic drugs, calcium channel blockers, cimetidine and omeprazole, metoclopramide, macrolide antibiotics, quinupristin/dalfopristin, danazol, ethinyl estradiol, methylprednisolone, and HIV protease inhibitors. Drugs that may decrease tacrolimus levels include some anticonvulsants (phenobarbital, phenytoin, carbamazepine), caspofungin, rifamycins, and St. John's wort.

Dose adjustments: The tacrolimus dose is adjusted to maintain a serum trough level of 5-15 ng/mL, with a target of 10-15 ng/mL. Patients with hepatic or renal insufficiency should receive doses at the lower end of therapeutic concentrations. No dose adjustments are required in patients undergoing hemodialysis.

Due to extreme interactions with voriconazole and posaconazole, the tacrolimus dose should be empirically lowered when these azoles are initiated at steady state levels of tacrolimus. Guidelines are provided in the table below section 8.17. Dose adjustments for therapy with other azoles may be indicated. However, the initial tacrolimus dose (on Day 5) remains fixed.

10.1.7 **Sirolimus**

Sirolimus is an immunosuppressant that inhibits cytokine-stimulated T-cell activation and proliferation, and also inhibits antibody formation.

Drug formulations: The mean bioavailability of sirolimus after administration of the tablet is ~27% higher than the oral solution. Sirolimus oral tablets are not bioequivalent to the oral solution. Clinical equivalence has been demonstrated at the 2-mg dose level; however, it is not known if higher doses are clinically equivalent on a mg to mg basis.

a) Sirolimus oral solution: Sirolimus oral solution (1 mg/mL) should be stored protected from light and refrigerated at 2°C to 8°C (36°F to 46°F). For dilution, the appropriate dose should be measured using an amber oral syringe, then added to a glass or plastic container that holds at least 60 mL. Before taking the dose, it should be diluted with water or orange juice then taken immediately; it should not be diluted with grapefruit juice. The syringe should be discarded after one use. Sirolimus oral solution provided in bottles may develop a slight haze when refrigerated, which does not affect product quality; allow the product to stand at room temperature and shake gently until the haze disappears.

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b) Sirolimus tablets: Sirolimus tablets are available in 1 mg and 2 mg tablets that cannot be crushed or broken. Sirolimus tablets should be stored at 20° to 25° C (68°–77°F), protected from light.

Toxicities: The most common adverse reactions of sirolimus are: peripheral edema, hypertriglyceridemia, hypercholesterolemia, hypertension, increased creatinine, constipation, abdominal pain, nausea, diarrhea, headache, fever, urinary tract infection, anemia, thrombocytopenia, arthralgia, pain. Adverse reactions that have resulted in rates of sirolimus discontinuation >5% were increased creatinine, hypertriglyceridemia, and thrombotic thrombocytopenic purpura (TTP) / thrombotic microangiopathy (TMA). Sirolimus toxicities are summarized in the table below:

Table3: Sirolimus toxicities

	Common (>20%)	Occasional (5-20%)	Rare (<5%)
Immediate (within 1-2 days)	Headache (L), hypertension (L), immunosuppression (L), fever, nausea, diarrhea, constipation	Chest pain, insomnia, dyspepsia, vomiting, dyspnea	Hypotension, asthma, cough, flu-like syndrome, tachycardia, anorexia, hypersensitivity reactions
Prompt (within 2-3 weeks)	Tremor (L), renal dysfunction, pain (abdominal, back, arthralgias), hyperlipidemia ^c (<i>hypercholesterolemia, hypertriglyceridemia</i>), hyperglycemia, edema including peripheral edema , anemia	Elevated LFT's (with elevated sirolimus levels) ^a , stomatitis, infections (including UTI, URI), mild thrombocytopenia , leukopenia , electrolyte disturbances (hyper/hypokalemia [L], hypophosphatemia, hypomagnesemia [L]), rash, hives, pruritus, delayed wound healing or dehiscence (L) , proteinuria , TTP/HUS/TMA ^b especially with concurrent CNI	Pleural and pericardial effusions, pulmonary toxicity (non-infectious pneumonitis, BOOP, pulmonary fibrosis), thrombosis, myalgias
Delayed (any time later during therapy, excluding above conditions)	Acne		Kidney disease, CHF, ascites, arthrosis, bone necrosis, osteoporosis
Late (any time after completion of treatment)			Lymphoproliferative disorders, skin malignancies
Unknown frequency and timing	Embryo/fetotoxic; unknown whether excreted in human milk		

(L): Toxicity may also occur later.

^a Significant transaminitis, generally without sequelae, may occur. Sirolimus has been associated with higher rates of venoocclusive disease after myeloablative conditioning.

^b Incidence 3% to < 20% in a trial of kidney transplantation. In allogeneic BMT, increase in TMA from 4.2% with tacrolimus or cyclosporine alone, versus 10.8% with tacrolimus/sirolimus combination was noted.⁶⁸

^c Lipid-lowering agent may be required; consider if fasting serum triglycerides are > 2.5 x ULN, and recommend starting if > 800 mg/dL.

Drug interactions: Sirolimus is known to be a substrate for both cytochrome CYP3A4 and P-glycoprotein. Agents that may increase sirolimus levels include tri-azole drugs (especially voriconazole and posaconazole*), amiodarone, calcium channel blockers, macrolide antibiotics (but not azithromycin), micafungin, gastrointestinal prokinetic agents (cisapride, metoclopramide), cimetidine, cyclosporine, grapefruit juice, and HIV protease inhibitors. Agents that may decrease sirolimus levels include anticonvulsants (carbamazepine, phenobarbital, phenytoin), rifamycins, St. John's Wort.

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Dose adjustments: The sirolimus dose is adjusted to maintain a serum trough level of 5-12 ng/mL. Changes in levels due to altered bioavailability should be apparent within 24-48 hours. For sirolimus without CNIs as in this study, a 20-25% reduction of sirolimus dose is recommended for trough levels >12 – 18 ng/mL, and a 20-25% increase is recommended for trough levels < 5 ng/mL.

Renal failure does not affect the excretion of sirolimus. Excretion is reduced in liver failure; impaired hepatic function should prompt consideration of reduction in sirolimus maintenance doses but no dose adjustment of the loading dose is necessary.

Due to extreme interactions with voriconazole and posaconazole, these drugs are relatively contraindicated during sirolimus therapy. Sirolimus dose is to be reduced by 90% when voriconazole is initiated and should also be significantly reduced with posaconazole. Dosing guidelines are provided in the table below.

Dosing considerations with concurrent azole therapy: Triazole antifungal medications are expected to increase serum CNI levels; therefore dosages of CNIs should be adjusted accordingly. Guidelines are provided in the table below. Of note, reversal of azole-mediated inhibition of CYP3A4 (and others) and P-glycoprotein is gradual when azoles are stopped. Therefore, immediate significant dose increases in tacrolimus and sirolimus are not advised when azoles are stopped. Rather, dose increases should be cautious and based on more frequent monitoring of levels as appropriate.

Table : Suggested preemptive dose reduction of tacrolimus when azoles are initiated at steady state levels of tacrolimus or sirolimus

Antifungal	Tacrolimus	
	Dose ↓	Comment
Voriconazole	67%	Strongly advised
Posaconazole	67%	Advised
Itraconazole	50%	Advised
Fluconazole	25%	Consider

10.2 Toxicity grading

Toxicities are graded using the NCI's Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm)

10.3 Toxicity reporting

The agents being used in the study are used extensively in the HSCT setting and have well-defined toxicity profiles. In addition, there are many expected toxicities of allogeneic HSCT. The following are examples of toxicities that are serious but not unexpected: Grade 4 cytopenias; neutropenic fever and sepsis; bacterial, fungal, or viral (including CMV, BK virus) infection; severe mucositis; severe GVHD; hepatic veno-occlusive disease; pulmonary toxicities; hemorrhagic cystitis; bleeding without hemodynamic compromise.

For study purposes, the following will be recorded and reported in accordance with IRB requirements:

- Any hospitalization and its reason in the first year of transplant, with the exception of hospitalizations related to relapsed disease or second HSCTs.
- Neutropenic fever is an expected, common complication; as such, hospitalizations for grade 4 neutropenic fever will be reported in real-time to the IRB with hospitalizations for lesser grade neutropenic fever routinely reported on a yearly basis.
- Any death before Day 200, and any later death which is potentially transplant-related.
- Any unexpected, serious events deemed significant by the PI.

In addition, the following toxicities will be tracked for study purposes and reported on a yearly basis to the IRB, or earlier if warranted:

- Clinically significant infections during the first year of transplant, with the exception of uncomplicated, culture-negative neutropenic fever. This includes CMV disease, other clinically significant documented viral infections, bacterial infections, and proven or probable invasive fungal infections.

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- b. CMV reactivation (including asymptomatic reactivation)
- c. Hepatic veno-occlusive disease
- d. Grade 3 or greater pulmonary toxicity during the first year of transplant that is potentially transplant-related

Additional complications and toxicities may be tracked. This is in addition to evaluating hematologic parameters, GVHD, and disease and survival endpoints outlined in Section 8.0.

11 Safety and Adverse Events

11.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others

Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc)
- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

Adverse Event

An **adverse event** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Serious Adverse Event

Adverse events are classified as serious or non-serious. A **serious adverse event** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as **non-serious adverse events**.

Adverse Event Reporting Period

The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 30 days following the last administration of study treatment.

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Preexisting Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event

All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an adverse event if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

Hospitalization, Prolonged Hospitalization or Surgery

Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should **not** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

11.2 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should be recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

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Pregnancy

Although pregnancy is not considered an AE, it is the responsibility of the investigator to report any pregnancy occurring in a female subject or female partner of a male subject, during the study. It must be reported immediately to the regulatory specialist, research coordinator, IRB, and NYUPCCsafetyreport@nyumc.org in accordance with the procedures described. Any complication of pregnancy affecting a female study subject or female partner of a male study subject, and/or fetus and/or newborn must be reported as an SAE. Every effort will be made to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus and/or newborn to NYULMC.

11.3 Reporting of Serious Adverse Events and Unanticipated Problems

Investigators and the protocol sponsor must conform to the adverse event reporting timelines, formats and requirements of the various entities to which they are responsible, but at a minimum those events that must be reported are those that are:

- related to study participation,
- unexpected, and
- serious or involve risks to subjects or others (see definitions, section 11.1).

For Narrative Reports of Safety Events

If the report is supplied as a narrative, the minimum necessary information to be provided at the time of the initial report includes:

- | | |
|------------------------------|--|
| • Study identifier | • Current status |
| • Study Center | • Whether study treatment was discontinued |
| • Subject number | • The reason why the event is classified as serious |
| • A description of the event | • Investigator assessment of the association between the event and study treatment |
| • Date of onset | |

11.3.1 Investigator reporting: NYULMC IRB and Perlmutter Cancer Center Clinical Trials Office

The following describes events that must be reported to the study sponsor in an expedited fashion.

Initial Report: within 24 hours:

The following events must be reported to the study sponsor by telephone within 24 hours of awareness of the event:

- Unanticipated problems related to study participation,
- Serious adverse events, regardless of whether they are unexpected.

Additionally, an FDA Form 3500A (MEDWATCH Form) must be completed by the investigator and faxed to the study sponsor within 24 hours. The investigator shall maintain a copy of the MEDWATCH Form on file at the study site.

NYUPCCsafetyreports@nyumc.org

AND

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Follow-up report: within 48 hours:

As a follow-up to the initial report, within the following 48 hours of awareness of the event, the investigator shall provide further information, as applicable, on the unanticipated device event or the unanticipated problem in the form of a written narrative. This should include a copy of the completed Unanticipated Problem form, and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing unanticipated adverse device effects shall be provided promptly to the study sponsor.

Other Reportable events:

- **Deviations from the study protocol**

Deviations from the protocol must receive both Sponsor and the investigator's IRB approval before they are initiated. Any protocol deviations initiated without Sponsor and the investigator's IRB approval that may affect the scientific soundness of the study, or affect the rights, safety, or welfare of study subjects, must be reported to the Sponsor and to the investigator's IRB as soon as a possible, but **no later than 5 working days** of the protocol deviation.

- **Withdrawal of IRB approval**

An investigator shall report to the sponsor a withdrawal of approval by the investigator's reviewing IRB as soon as a possible, but **no later than 5 working days** of the IRB notification of withdrawal of approval.

11.3.2 Investigator reporting: notifying the IRB

Federal regulations require timely reporting by investigators to their local IRB of unanticipated problems posing risks to subjects or others. The following describes the NYULMC IRB reporting requirements, though Investigators at participating sites are responsible for meeting the specific requirements of their IRB of record.

The NYU IRB address is:

NYU IRB
1 Park Avenue, 6th Floor
New York, NY 10016

Report Promptly, but no later than 5 working days:

Researchers are required to submit reports of the following problems promptly but no later than 5 working days from the time the investigator becomes aware of the event:

- **Unanticipated problems including adverse events that are unexpected and related**

- *Unexpected: An event is "unexpected" when its specificity and severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.*
- *Related to the research procedures: An event is related to the research procedures if in the opinion of the principal investigator or sponsor, the event was more likely than not to be caused by the research procedures.*
- *Harmful: either caused harm to subjects or others, or placed them at increased risk*

Other Reportable events:

The following events also require prompt reporting to the IRB, though **no later than 5 working days**:

- **Complaint of a research subject** when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- **Protocol deviations or violations** (includes intentional and accidental/unintentional deviations from the IRB approved protocol) for any of the following situations:
 - *one or more participants were placed at increased risk of harm*
 - *the event has the potential to occur again*
 - *the deviation was necessary to protect a subject from immediate harm*

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- **Breach of confidentiality**
- **Incarceration of a participant** when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- **New Information indicating a change to the risks or potential benefits** of the research, in terms of severity or frequency. (e.g. analysis indicates lower-than-expected response rate or a more severe or frequent side effect; Other research finds arm of study has no therapeutic value; FDA labeling change or withdrawal from market)

Reporting Process

The reportable events noted above will be reported to the IRB using the form: "Reportable Event Form" or as a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution and need for revision to consent form and/or other study documentation).

Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's study file.

11.3.3 Sponsor reporting: *Notifying the FDA*

11.3.4 Sponsor reporting: *Notifying participating investigators*

N/A

11.4 *Unblinding Procedures*

N/A

11.5 *Stopping Rules*

N/A

11.6 *Medical Monitoring*

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan detailed below. Adverse events are evaluated regularly by the principal investigator in conjunction with the research team; the DSMC reviews these adverse events monthly. The Data Safety and Monitoring Committee (DSMC) will review the study at least annually. Medical monitoring will include a regular assessment of the number and type of serious adverse events.

11.6.1 *Data and Safety Monitoring Plan*

This investigator-initiated study will be monitored by the Data Safety Monitoring Committee (DSMC) of the New York University (NYU) Perlmutter Cancer Center (PCC). The DSMC operates based on the 2014 National Cancer Institute approved Charter. It is an existing and multidisciplinary committee (consisting of clinical investigators/oncologists, biostatisticians, nurses, and research administration staff knowledgeable of research methodology and design and in proper conduct of clinical trials) that is responsible for monitoring safety, conduct and compliance in accordance with protocol data monitoring plans for clinical trials conducted in the NYULMC Perlmutter Cancer Center that are not monitored by another institution or agency. The DSMC reports to the Director of the NYULMC PCC.

Per the NYU PCC Institutional Data Safety and Monitoring Plan, this phase II trial will be monitored by DSMC annually (from the date the first patient is enrolled) and at the completion of the study prior to study closure. This review includes accrual data, subject demographics and adverse events. Principal Investigators are required to attend the review of their studies. Additional reviews can be scheduled based on SAE reports, investigator identified issues, external information, etc. The DSMC will review safety data annually.

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12 Data Handling and Record Keeping

12.1 Confidentiality

The research team will maintain clinical and laboratory data in a manner that ensures patient confidentiality. All study personnel have passed human subject protection courses and GCP. Tissue samples sent to collaborators outside of NYU PCC will only be labeled with an assigned protocol subject identification number without patient identifiers. Systems used for electronic data capture are compliant with HIPAA and applicable local regulatory agency guidelines. All documents are kept in strictly confidential files and are only made accessible for specific study personnel, CTO quality assurance specialists, and authorized representatives of regulatory agencies as described in the informed consent document. Samples sent to commercial labs or collaborating labs as per study protocol will be coded. Samples remaining after completion of the study will be destroyed once this study is completed.

12.2 Confidentiality and HIPAA

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

12.3 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Source documentation refers to original records of observation, clinical findings, and evaluations that are subsequently recorded as data. Source documentation should be consistent with data entered into Velos. Relevant source documentation to be reviewed by the DSMC throughout the study includes:

1. Baseline measures to assess pre-protocol disease status
2. Treatment records
3. Concomitant medications
4. Adverse events

12.4 Data and Source Documentation

Velos, an electronic database capture system will be created to record the data for this trial. Research coordinators will input clinical trial data into the database. This database is password protected and only the PI, assigned research coordinator, and CTO quality assurance specialists will have access to the database. Velos is the primary data collection instrument for the study. All data requested in Velos must be reported. All missing data must be explained. The quality assurance specialists will begin Interim monitoring visits within the first 6-8 weeks of first subject enrollment and then monitor this trial every 6-8 weeks thereafter for data entry and accuracy.

12.5 Records Retention

All patient records will be maintained for at least 10 years according to NYULMC policy. Of note, all data collected in this study is part of the patient's standard medical care and will be maintained in his/her medical record.

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13 Study Monitoring, Auditing, and Inspecting

13.1 Study Monitoring Plan

This study will be monitored according to the monitoring plan detailed below. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit. A risk-based, data-driven monitoring approach will be used to verify data for this trial which will also include a centralized review of data for quality, trends, consistency and general safety review. A quality assurance specialist will make regularly scheduled trips to the investigational site to review the progress of the trial, study data and site processes. At each visit, the monitor will review various aspects of the trial including, but not limited to: screening and enrollment logs; compliance with the protocol and study manual and with the principles of Good Clinical Practice; completion of case report forms; source data verification; study drug accountability and storage; facilities and staff.

During scheduled monitoring visits, the investigator and the investigational site staff must be available to meet with the quality assurance specialist in order to discuss the progress of the trial, make necessary corrections to case report form entries, respond to data clarification requests and respond to any other trial-related inquiries of the monitor. In addition to on-site monitoring visits, the Sponsor and/or representatives will also be routinely reviewing data. Any queries identified through this review will be managed within the systems established for query resolution and tracking. Inquiries related to study conduct, which require further information or action will be discussed within the study team for appropriate and documented escalation plans. It is expected that response to data clarification requests and other trial-related inquiries will occur throughout the course of the study through regular communication with the site monitor, the Sponsor or representatives, and review/entry of data into the electronic study database.

At any time during the course of the study, representatives of the FDA and/or local regulatory agencies may review the conduct or results of the study at the investigational site. The investigator must promptly inform NYU PCC CTO of any audit requests by health authorities..

In accordance with HIPAA and associated privacy regulations, a patient's authorization to use personal identifiable health information may be required from each patient before commencement of research activities. This authorization document must clearly specify what parties will have access to a patient's personal health information, for what purpose and for what duration.

At the NYU Perlmutter Cancer Center, all investigator-initiated protocols are subject to a standardized data and safety monitoring, which includes scientific peer review, IRB review and DSMC review as well as internal auditing.

The review of AEs and trial conduct for this trial occurs at several levels:

- (1) Principal Investigator: Adverse events are evaluated monthly by the principal investigator in conjunction with the research nurses, data manager and research team.
- (2) DSMC, annually
- (3) Institutional Review Board (IRB): An annual report to the IRB is submitted by the trial PI for continuation of the protocol. It includes a summary of all AEs, total enrollment with demographics, protocol violations, and current status of subjects as well as available research data.
- (4) In addition, the quality assurance unit will monitor this trial by quarterly interim monitoring visits, to verify adherence to the protocol; the completeness, accuracy and consistency of the data; and adherence to ICH Good Clinical Practice guidelines.

13.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB/EC, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

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Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices. The investigator will contact the PCC CTO immediately if contacted by a regulatory agency about an inspection at the center.

14 Ethical Considerations

This study is to be conducted accordance with applicable US government regulations and international standards of Good Clinical Practice, and applicable institutional research policies and procedures.

This protocol and any amendments will be submitted to the NYU Institutional Review Board (IRB) in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of IRB members and their affiliate to the sponsor.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

The consenting process and documentation will follow Standard Operating Procedures (Obtaining Informed Consent for Clinical Trials) of the NYULMC PCC CTO.

15 Study Finances

15.1 Funding Source

All drugs, nursing, and physician visits are considered part of standard care and will be covered by the patient and/or his/her insurance. Data collection and analysis for this study will be funded by NYULMC and Perlmutter Cancer Center sources.

15.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All NYULMC investigators will follow the applicable University conflict of interest policies.

15.3 Subject Stipends or Payments

No patient or subject will receive payments or stipends for participation in this research study.

16 Publication Plan

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

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18 Appendix

Appendix A: Acute GVHD Grading

Clinical Staging

Stage	Skin	Liver: Total Bilirubin	Intestinal Tract: Diarrhea
0	No rash	<2.0 mg/dL	<500 ml/day
1	<25% of skin surface	2.0-3.0	500-1000 ml/day
2	25-50%	3.1-6.0	1001-1500 ml/day
3	Erythroderma	6.1-15.0	>1500 ml/day
4	Erythroderma with bullae and desquamation	>15.0	Severe abdominal pain with or without ileus

Clinical Grading

Grade	Skin*	Liver	GI
I	1-2	0	0
II	3	1	1
III	-	2-3	2-4
IV	4	4	-

*Each column identifies minimum stage for organ grade

From Przepiorka D et al. 1994 Consensus Conference on Acute GVHD Grading. HSCT 1995; 15: 825-828.

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